# β-Sultams—Mechanism of Reactions and Use as Inhibitors of Serine Proteases

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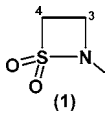
**ABSTRACT**

β-Sultams are reactive sulfonyl analogues of β-lactams and show enormous rate enhancements over analogous reactions of sulfonamides. N-Acyl β-sultams undergo S−N rather than C−N fission, although α-alkenyl substituents direct nucleophilic attack to the acyl center. They also inactivate serine enzymes such as elastase and β-lactamase by sulfonylation of the active site serine. Structure−activity relationships are used to identify differences in transition state structures.

## Introduction

β-Sultams, **1**, are the sulfonyl analogues of β-lactams, **2**, and are potential sulfonylating agents of a variety of



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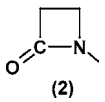

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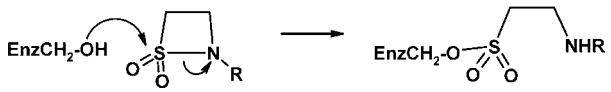
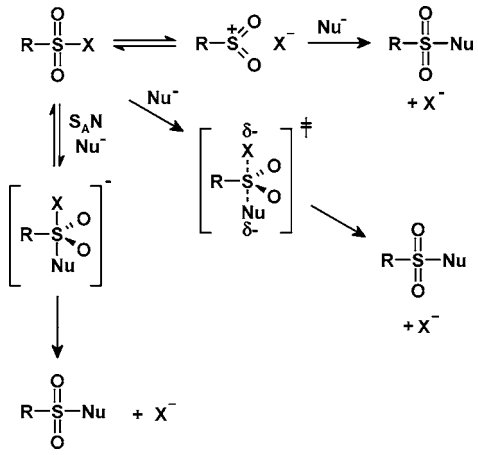
nucleophiles. As sulfonamides, albeit in cyclic four-membered rings, β-sultams also have the potential to act as peptide mimics and as transition state analogues of the tetrahedral intermediates formed in many acyl transfer reactions. However, compared with acyl transfer, the mechanisms of sulfonyl transfer reactions have been much less well studied.[1] We have been interested in β-sultams both as possible inhibitors of proteolytic enzymes and as reactive sulfonyl derivatives capable of yielding useful mechanistic information for comparison with the more extensively studied β-lactams.[2] β-Lactams are well-known inhibitors of DD-transpeptidase, β-lactamase, elastase, and other serine proteases by acting as acylating agents of the active site serine.[3] It is of interest to compare this activity with that of β-sultams, which could act as sulfonylating inhibitors of serine proteases (Scheme 1). The mechanisms of acyl transfer reactions commonly involve stepwise nucleophilic addition and expulsion of the leaving group and generally appear not

Scheme 1



Scheme 2



to be concerted.[1] Conversely, the mechanisms of sulfonyl transfer are usually discussed in terms of a concerted displacement, and evidence for a stepwise process is often ambiguous.[4] The stereochemical and geometrical requirements for substitution at four-coordinate sulfonyl centers are also inherently different from those at three-coordinate acyl centers. Any sulfonyl transfer reaction catalyzed by proteases therefore may give interesting insights into the flexibility of enzymes and requirements for the precise alignments of atoms undergoing bond-making and bond-breaking.

Possible mechanisms for sulfonyl transfer are shown in Scheme 2. They include the dissociative, $S_N1(S)$-type, process, which would generate a sulfonylium ion intermediate that is subsequently attacked by a nucleophile. However, the evidence for this mechanism is ambiguous, and it appears that sulfonylium ions are much more difficult to generate than acylium ions.[4] Also shown in Scheme 2 are associative mechanisms, which are presumed to involve a five-coordinate trigonal bipyramidal geometry around sulfur in either the transition state or intermediate in the concerted or stepwise processes, respectively. There is considerable controversy concerning the timing of bond-making and bond-breaking in the associative mechanism. The use of linear free-energy relationships to differentiate stepwise or concerted processes is not free from criticism and has, in fact, been used to support both mechanisms;[4,5] most observations can be interpreted in terms of either a stepwise or concerted mechanism depending on the prejudices of the authors.

Acyclic sulfonamides are extremely resistant to alkaline and acid hydrolysis.[4] The NH acidity of sulfonamides is greater than that of carboxylic acid amides, and the $pK_a$ of sulfonamides is typically around 10−11 so that they are fully ionized in alkaline solution. However, formation of the anion is not the sole reason for the lack of reactivity because sulfonamides of secondary amines are also un-

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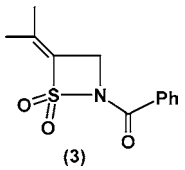
reactive. The difficulty of acid-catalyzed hydrolysis arises from the low basicity of sulfonamides, which is reflected in the apparent p$K_a$'s of their conjugate acids of about −6. They are thus less basic than carboxamides and also differ by undergoing protonation on nitrogen.[6] The indications are that the sulfonyl group is more electron-withdrawing than an acyl center, but there is little evidence for delocalization of the nitrogen lone pair onto the sulfonyl oxygen atoms in sulfonamides.

## Structure of β-Sultams

β-Sultams are formally 1,2-thiazetidine 1,1-dioxides, **1**, and are nonplanar or planar depending on substituents. The nitrogen atom in N-alkyl β-sultams is generally pyramidal, and the nitrogen is 0.4−0.7 Å out of the plane defined by $S_1C_3C_4$.[7−9] In β-lactams, the nitrogen ranges from being essentially in the plane of its three substituents in monocyclic β-lactams to being 0.6 Å out of the plane in bicyclic systems such as penicillins and carbapenems.[2] Exocyclic N-acylation of β-sultams converts the ring nitrogen to an amide and consequently the nitrogen becomes less pyramidal or even coplanar with the ring atoms. However, the introduction of an unsaturated substituent in the ring such as in 4-alkenyl-N-acyl β-sultams, for example, **3**,



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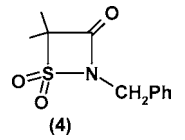
forces the acylated nitrogen to become more pyramidal.[8,9] In contrast, the 3-oxo-β-sultam **4** adopts an almost planar



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structure.[10] In general, the internal bond angle around sulfur is 79° ± 1° compared with 113° in acyclic sulfonamides, while that around nitrogen is 95° ± 1° irrespective of whether the nitrogen substituent is alkyl or acyl. Finally, the S−N bond length of 1.70 Å compared with that of 1.52 Å for $C_3C_4$ and the longer S−$C_4$ than $C_3$−N bond length gives the β-sultam an interesting overall geometry. There is thus considerable ring strain in β-sultams as a result of bond angle strain. It is unlikely that there is an additional influence of strain resulting from loss of resonance energy because of the constraint of the nitrogen and the sulfonyl centers within a four-membered ring, and in any case, there is little evidence to suggest that sulfonyl groups stabilize adjacent atoms with lone pairs by resonance.[11]

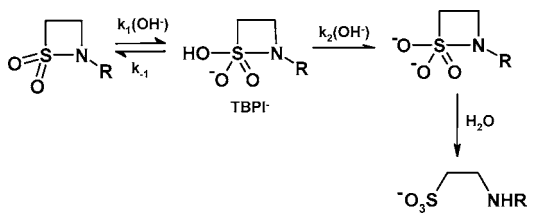
## Hydrolysis/Reactivity

The hydrolysis of β-sultams normally occurs with exclusive S−N fission to give the corresponding β-aminosulfonic acid.[8] Of particular note is the very high reactivity of β-sultams toward acid and base hydrolysis compared with other sulfonamides.[8] Acyclic sulfonamides are so unreactive toward alkaline hydrolysis that it is difficult to measure the rate constants accurately, but β-sultams are estimated to be at least $10^7$-fold more reactive.[12] This is in sharp contrast to the almost identical rate of alkaline hydrolysis of β-lactams compared with that of their acylic amide analogues.[2] It is a surprising fact that the strain energy inherent in the four-membered ring of β-lactams is not even partially released in the transition state to lower the activation energy for reaction. In general, sulfonyl transfer reactions occur $10^2$−$10^4$-fold more slowly than the corresponding acyl transfer process,[13] yet β-sultams are $10^2$−$10^3$-fold *more* reactive than corresponding β-lactams. This appears to be the first example of the rate of sulfonyl transfer being greater than that of the corresponding acyl reaction. Attack by hydroxide ion on the β-sultam sulfur must be accompanied by a large relief in ground-state bond angle strain upon formation of the transition state. There are several indications that there is considerable S−N bond fission in the transition state for ring opening reactions of β-sultams, so the strain energy of the four-membered ring is partially released in the transition state.[8]

## Alkaline Hydrolysis and Evidence for a Trigonal Bipyramidal Intermediate (TBPI)

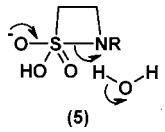
Direct substitution at sulfonyl sulfur is thought to occur with inversion of configuration.[14] Although this indicates that the geometry of the displacement probably involves a transition state in which the entering and leaving groups occupy the two apical positions of a trigonal bipyramid, it does not distinguish between a $S_N2$-type transition state and an intermediate with a significant lifetime. In fact, there is no unambiguous evidence for the existence of a trigonal bipyramidal intermediate in sulfonyl transfer.[4] However, rates of the alkaline hydrolysis of some β-sultams are second-order in hydroxide ion, which is strong evidence for the formation of a trigonal bipyramidal intermediate (TBPI) with a hypervalent sulfur.[15] Initial but *reversible* attack of hydroxide ion on the β-sultam generates a monoanionic TBPI$^-$, which requires deprotonation by a second hydroxide ion before the intermediate can collapse to products (Scheme 3).[8]

**Scheme 3**




There is a difference in the timing of the proton transfer and possibly rate-limiting steps for the alkaline hydrolysis of N-aryl and N-alkyl β-sultams. The β-sultams of anilines are more reactive than those of alkylamines toward alkaline hydrolysis by more than one 100-fold, reflecting the difference in basicity between anilines and alkyl-

amines. The rates of hydrolysis of N-aryl β-sultams increase with electron-withdrawing substituents in the aromatic ring and generate a Bronsted $\beta_{lg}$ of −0.58. This and a kinetic solvent isotope effect (KSIE) of 0.60 are consistent with rate-limiting formation of the anionic trigonal bipyramidal intermediate, $TBPI^-$.[8] The KSIE for N-alkyl derivatives is 1.6 indicating that the rate-limiting step is different for the more basic amine leaving group. Breakdown of $TBPI^-$ by S−N fission almost certainly requires protonation of the nitrogen of alkylamines, and the most likely mechanism for the alkaline hydrolysis of β-sultams of alkylamines is rate-limiting ring opening facilitated by partial proton transfer from water, **5**.[8] Rate-



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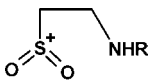
limiting opening of the strained β-sultam ring appears to be yet another example of the relative difficulty of bond cleavage in four-membered rings despite the release of strain energy. This unexpected phenomenon has been previously observed in azetidine derivatives and has been linked to the detailed mechanics of ring opening, which may occur by bond rotation rather than bond stretching.[16]

## Acid Hydrolysis and Evidence for a Sulfonylium Ion Intermediate

β-Sultams undergo an acid-catalyzed hydrolysis, but in contrast to alkaline hydrolysis, N-alkyl β-sultams are more reactive in acid than the N-aryl derivatives. Acyclic sulfonamides are believed to undergo N-protonation,[6] and if the same is true of β-sultams, the less basic nitrogen in N-aryl β-sultams will result in less favorable protonation and a reduced rate of hydrolysis. Preequilibrium protonation of the β-sultam nitrogen facilitates S−N bond fission by allowing the amine leaving group to depart as the neutral amine. The combination of this with the relief of ring strain permits the possible involvement of a unimolecular A1 process to form an electron-deficient sulfonylium ion intermediate **6**, which could then be



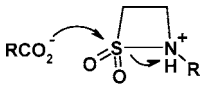
(6)

trapped by water to form the β-aminosulfonic acid product. Electron-withdrawing substituents α to the sulfonyl group have a very large retarding effect upon the rate of acid-catalyzed hydrolysis and generate a very negative Hammett $\rho_I$ value.[15] These observations are compatible with a unimolecular process to form the sulfonylium ion **6**. There are no well-established cases for these intermediates during substitution at sulfonyl centers,[4] but their intermediacy in β-sultam hydrolysis is compatible with the similar acylium ion mechanism suggested for the acid-catalyzed hydrolysis of β-lactams.[17]

## Reactions of β-Sultams with Nucleophiles

The kinetics and mechanisms of nucleophilic substitution reactions at sulfonyl centers have not been extensively studied because of the generally low reactivity of common sulfonylated compounds.[1,4] The relative reactivities of β-sultams with O, S, and N nucleophiles is of interest in connection with the use of β-sultams as potential sulfonylating agents of enzymes.[18,19] Surprisingly, and in contrast to the less reactive β-lactams, many of the β-sultams do not react readily with nucleophiles other than hydroxide ion in aqueous solution, and their ability to do so is controlled by the nature of the leaving group such that the amine nitrogen needs to be either fully protonated or to be able to leave as an anion.

The rate of hydrolysis of N-benzyl β-sultam is increased by carboxylic acid buffers at constant pH[8] and dependent on the concentration of the undissociated carboxylic acid. The observation of general *acid*-catalyzed hydrolysis contrasts with the general *base* catalysis seen with the buffer-catalyzed hydrolysis of β-lactams of penicillins.[20] Although referred to as "general acid catalysis", the probable mechanism of reaction involves specific acid−nucleophilic catalysis.[8,21] The β-sultam undergoes reversible protonation on nitrogen, followed by direct nucleophilic attack of the carboxylate anion **7** to form a mixed



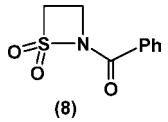
(7)

acid anhydride intermediate, which is subsequently hydrolyzed or it may be trapped with aniline to give acetanilide.[21] The direct displacement of the strongly basic amine by a weakly basic carboxylate anion is unusual. No such reaction is seen with penicillins where carboxylate anions act as general base catalysts and facilitate the attack of water on the β-lactam,[20] but the protonation of nitrogen in the β-sultam obviously enhances the ease of S−N fission.

N-Alkyl and N-aryl β-sultams do not show any reaction with amines or thiols or even oxygen nucleophiles in aqueous solutions above neutral pH, other than with hydroxide ion. This again is in sharp contrast to the β-lactams of penicillins and cephalosporins where these reactions occur readily in competition with hydrolysis.[2,22] The rate of alkaline hydrolysis of N-benzyl β-sultam is about 10-fold less than that of benzyl penicillin, whereas N-phenyl β-sultam is 50-fold more reactive. Why are N, S, and O nucleophiles not able to compete with $HO^-$ in attacking N-aryl β-sultams, which should be good sulfonating agents? In general, sulfonyl centers are much less reactive than analogous acyl centers toward nucleophiles, with the exception of the sulfonyl halides.[12,23] However, β-sultams are more reactive than their acyl analogues, the β-lactams. The β-sultams are unusual sulfonyl compounds as other sulfonyl derivatives such as benzenesulfonyl chloride, which shows a reactivity toward hydroxide similar to that for N-aryl β-sultams, readily undergoes

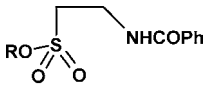
aminolysis in water.[24] That nucleophiles cannot compete with hydroxide ion reacting with N-alkyl and N-aryl β-sultams may simply be due to a relatively enhanced rate of hydrolysis by hydroxide ion as sulfonyl compounds show a preference toward oxygen nucleophiles.[25]

However, selectivity between nucleophiles is not just a matter of reactivity because the N-benzoyl β-sultam **8**




(8)

is an extremely reactive β-sultam and is 300-fold more reactive toward hydroxide ion than is N-*m*-chlorophenyl β-sultam and yet *does* undergo reaction with nucleophiles in water, although only readily with O-nucleophiles, such as alcohols and carboxylate anions.[26] The reaction between oxyanions and the β-sultam **8** involves nucleophilic substitution at the sulfonyl center. With weakly basic oxygen nucleophiles, the reaction involves catalyzed hydrolysis as the intermediate sulfonate ester **9** undergoes




(9)

rapid hydrolysis to the sulfonic acid, while with basic nucleophiles, alcoholysis occurs as the sulfonate ester is relatively stable.[26] The rates of nucleophilic substitution increase with the basicity of the oxygen nucleophile and give a Bronsted $\beta_{nuc}$ of +0.6 consistent with either stepwise rate-limiting formation of the TBPI or a concerted mechanism of ring opening.[26] The reactivity order for N-benzoyl β-sultam **8** toward nucleophiles is $HO^- > RO^- > F^- > RCO_2^- > RNH_2 > H_2O$. This low reactivity of amines is also observed in the reactions of *p*-nitrophenyl toluenesulfonate where there is a distinct preference toward oxygen nucleophiles.[27] In contrast to acyl esters, N-acyl β-sultams show a larger degree of selectivity the more reactive the compound consistent with an inverse selectivity−reactivity relationship for sulfonyl compounds.[12,24,26] For sulfonyl halides and N-acyl β-sultams, the more reactive compounds are apparently more selective. The reactivity order of nucleophiles toward β-sultams indicates that the sulfonyl center is a hard electrophile.

## C−N versus S−N Fission in N-Acylsulfonamides

N-Acylsulfonamides normally react with nucleophiles to give N-acyl fission as a result of nucleophilic attack on the carbonyl group followed by displacement of the sulfonamide anion. In so far as sulfonamides are stronger acids than amides by about 5 p*K* units, sulfonamide anions are usually better leaving groups than amide anions. The hydrolysis of N-acyl β-sultams, for example, **8**, may involve either ring opening, arising from nucleophilic attack of hydroxide ion upon the sulfonyl center and expulsion of an amide leaving group, or attack of hydroxide ion upon the exocyclic acyl amide group leading to amide hydrolysis and preservation of the β-sultam ring. Normal N-acylsulfonamides behave as reactive amides and undergo C−N fission with rates of alkaline hydrolysis up to $10^5$ faster than "normal" amides.[28] By contrast, the alkaline hydrolysis of N-benzoyl β-sultam, **8**, occurs exclusively by S−N fission as a result of attack on sulfur and displacement of the carboxamide.[9] This is the first example of hydrolysis of a N-acylsulfonamide occurring with S−N rather than C−N fission. N-Benzoyl β-sultam, **8**, is $10^4$-fold more reactive than an analogous acyclic N-acylsulfonamide occurring by C−N fission.

Although N-acyl β-sultams generally undergo hydrolysis with S−N fission and displacement of the amide group,[26] the steric demands of sulfonyl and acyl transfer differ and α-substituents can redirect the mode of preferential attack.[9] 4-Isopropylidene β-sultam, **3**, undergoes alkaline hydrolysis about $10^4$-fold more slowly than the unsubstituted N-benzoyl β-sultam, **8**. Product analysis shows that hydroxide ion attacks the exocyclic carbonyl group of **3** leading to C−N fission and formation of the intact β-sultam and benzoic acid.[9] The α-isopropylidene substituent must decrease the rate of nucleophilic substitution at the sulfonyl center by at least $10^6$-fold compared with that in **8**. This is an extraordinarily large factor and may be compared with the negligible effect of α-alkenyl substitution at acyl centers. Attack at the sulfonyl center in **3** is precluded by the unfavorable interaction between the syn methyl group of isopropylidene and the incoming hydroxide ion, which must increase the activation energy so much that it becomes larger than that required for attack at the acyl center.[9]

The incorporation of the acyl center within the four-membered ring gives a structure, **4**, which is both a β-sultam and a β-lactam.[28] These 3-oxo β-sultams also undergo hydrolysis with preferential attack on the sulfonyl center leading to S−N fission. Despite the presumably very large strain in 3-oxo β-sultams, **4** is only 10-fold more reactive toward alkaline hydrolysis than the β-sultam with an exocyclic acyl center.[28] That hydroxide ion attacks the sulfonyl center in **4** rather than the β-lactam carbonyl is consistent with the observation that β-sultams are more reactive than β-lactams toward alkaline hydrolysis. However, attack at the acyl center would expel a better leaving group, the sulfonamide anion, than attack at the sulfonyl center to expel the amide anion. On the basis of the similar reactivities of imides and N-acylsulfonamides toward alkaline hydrolysis, the nature of the leaving group does not have a large effect.[28]

## Inhibition of Serine Proteases by Sulfonylation using β-Sultams

Proteolytic enzymes are potential therapeutic targets for drug action, and their inhibition is a fruitful area of study. In general, this inhibition involves the covalent modification of an active site residue, which is not then readily regenerated. For example, many inhibitors are acylating agents of the active site serine residue of serine pro-

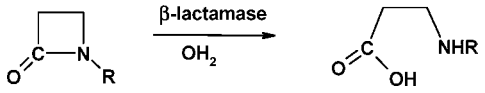
teases.[29] The mechanism of inhibition involves the displacement of a leaving group from the acylating agent to generate a relatively stable acylenzyme, which only reacts slowly with nucleophiles, such as water, to regenerate the enzyme, so this process leads to effective inhibition. The sulfonylation of serine proteases (Scheme 1) offers an interesting but largely unexplored strategy for inhibition because sulfonyl derivatives are much less reactive than their acyl counterparts.[13]

Human neutrophil elastase (HNE) is a serine enzyme and is one of the most destructive proteolytic enzymes capable of catalyzing the hydrolysis of components of connective tissue. It has been implicated in the development of diseases such as emphysema, cystic fibrosis, and rheumatoid arthritis, and there have been numerous studies attempting to find small molecule inhibitors of HNE. β-Lactams, traditionally used as antibacterial agents by inhibiting serine transpeptidases, have also been shown to be mechanism-based inhibitors of elastase when used as neutral derivatives.[30] Electrospray ionization mass spectrometry (ESI-MS) and NMR studies have shown that the first step is an acylation process in which the four-membered β-lactam ring is opened,[30] and we have explored the analogous reaction with β-sultams (Scheme 1).

N-Acyl β-sultams are time-dependent inhibitors of elastase, and enzyme activity decreases irreversibly in a first-order rate process giving rate constants dependent on inhibitor concentration.[18] The corresponding second-order rate constants for inactivation, $k_i$, vary with pH in a manner similar to that for the hydrolysis of an anilide substrate ($k_{cat}/K_m$) catalyzed by elastase. This indicates that the rate of inactivation of elastase by β-sultams is controlled by the same catalytic groups in the active site that are used for substrate hydrolysis, that is, active-site-directed inhibition is occurring. The increase in enzyme activity toward inactivation with increasing pH shows an apparent p$K_a$ of about 7 and is probably due to the dissociation of the protonated His-57 residue, crucial for effective catalysis of the substrate residue.[19] Covalent modification of the enzyme by sulfonylation was confirmed by ESI-MS; native elastase has a $M_r$ = 25 904 Da, but incubation of the enzyme with N-benzoyl β-sultam (**8**) (MW = 211 Da) with elastase showed formation of the sulfonylated adduct at 26 115 Da. Furthermore, X-ray crystallography of the inactivated enzyme shows ring opening of the β-sultam and formation of a sulfonate ester of Ser-195 (Scheme 1). One of the sulfonate oxygen atoms is located in the oxyanion hole, while the other occupies the upper part of the $S_1$ pocket.[18]

N-Acylsulfonamides have been used previously to inactivate serine enzymes,[31] but the mechanism invariably involves acylation and C−N bond fission with the serine hydroxyl group attacking the amide to displace the sulfonamide as the leaving group. The inactivation of elastase by N-acyl β-sultams appears to be the first case of preferential S−N over C−N fission in the reaction of a N-acylsulfonamide with a serine protease.[18] Furthermore, enzyme-catalyzed sulfonylation indicates a significant degree of flexibility within the protein as the stereochemical requirements for catalysis of a reaction involving sulfonyl transfer with a trigonal bipyramidal arrangement in the transition state are significantly different from acyl transfer reactions involving a tetrahedral intermediate. Although C−N bond fission in the hydrolysis of amides requires general acid catalysis to facilitate amine expulsion, the reactivity of N-acyl β-sultams and their amide leaving groups may allow ring opening to occur without N-protonation.

**Scheme 4**



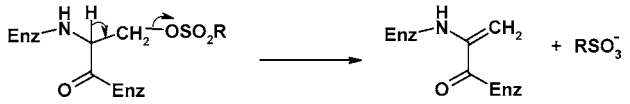

**Scheme 5**


The rates of enzyme inactivation can be increased if recognition elements are built into the structure of the β-sultams by improving binding to elastase and reducing the hydrolytic lability of the inhibitor. Elastase has a binding pocket adjacent to the active serine residue, which is relatively small and has a preference for small hydrophobic substituents.[19] The introduction of an isopropyl substituent at the 4-position of the thiazetidine ring lowers the reactivity of β-sultams toward hydroxide ion by 50-fold, whereas enzyme inhibitory activity is only decreased by 3-fold, indicative of favorable interactions between the alkyl substituent and the hydrophobic binding pocket.[19] Variation in the structure of 4-alkyl and N-substituted β-sultams causes differences in the rates of inactivation by 4 orders of magnitude. Such structure−activity relationships highlight the possibilities for further development of this series of compounds to enhance the selectivity of enzyme inhibition.

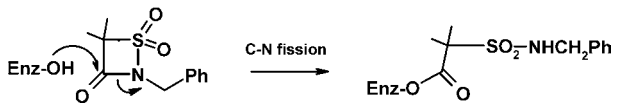
The susceptibility of β-lactam antibiotics to the hydrolytic activity of β-lactamase enzymes is the most common and growing form of bacterial resistance to the normally lethal action of these antibacterial agents.[32] β-Lactamases catalyze the hydrolysis of the β-lactam to give the ring-opened and bacterially inert β-amino acid (Scheme 4). Bacteria are producing new β-lactamases that can catalyze the hydrolysis of β-lactams previously resistant to enzyme degradation. For example, when carbapenems, such as imipenem, were first introduced in the 1970s, they were seen as versatile broad-spectrum antibacterials resistant to hydrolysis by most β-lactamases. However, "carbapenamases" capable of cleaving these derivatives are now increasingly produced by a variety of bacteria.[32]

The main mechanistic division of β-lactamases is into serine and zinc enzymes. The former have an active site serine residue, and the catalytic mechanism involves the formation of an acyl-enzyme intermediate[32] (Scheme 5). The class C β-lactamases of Gram-negative bacteria are widely expressed and are not significantly inhibited by clinically used β-lactamase inhibitors such as clavulanic

**Scheme 6**



**Scheme 7**



acid. It has been suggested that the phenol of tyrosine 150 in class C β-lactamase has a severely reduced p$K_a$ of 6.3, which acts as a general base catalyst for proton removal from serine 64 (Scheme 5).[33] In common with serine proteases, the serine β-lactamases possess an oxyanion hole, which donates two hydrogen bonds to the β-lactam carbonyl oxygen, inducing polarization to facilitate nucleophilic attack and stabilizing the oxyanion of the tetrahedral intermediate.

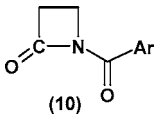
P99 β-Lactamase is a serine class C β-lactamase enzyme derived from the Gram-negative bacteria *Enterobacter clocae* with a molecular weight of 39 204 Da and is capable of hydrolyzing a wide variety of β-lactam-based substrates. N-Benzoyl β-sultam, **8**, is a time-dependent inhibitor of P99 β-lactamase, and enzyme activity decreases with time in an exponential manner to give apparent pseudo-first-order rates of inactivation.[34] The rates of inactivation of P99 β-lactamase by the β-sultam **8** show a similar sigmoidal dependence on pH to that for the hydrolysis of cephaloridine as substrate. Both rates depend on a catalytic group in the enzyme that ionizes with a p$K_a$ of 6.3, which is good evidence for active-site-directed inhibition. The presence of an excess of the tight-binding substrate benzyl penicillin retards the rate of inhibition by β-sultams indicating that the β-sultam is reacting at the active site. ESI-MS of solutions of P99 β-lactamase incubated with the β-sultam **8** reveals the enzyme bound to 1 equiv of β-sultam (MW 39 382 Da). The mass difference of +216 is consistent with the sulfonylation of the active serine residue Ser-64 to form a stable and inactive sulfonyl enzyme.[34] However, over a period of a few hours, the mass slowly returns to that of the native enzyme less 18 (MW 39 148(4) Da) suggesting that, after sulfonylation of the enzyme, the O-sulfonylated serine undergoes an elimination to give dehydroalanine (Scheme 6). Enzyme inactivation remains irreversible, and there is no return of enzyme activity over 4 days. This elimination reaction does not occur with elastase inactivated by the same β-sultam **8**.

Selectivity between the inhibition of β-lactamase and elastase by β-sultams is demonstrated by introduction of a 4-isopropyl substituent, which decreases the rate of inactivation by over $10^3$ for β-lactamase and makes this derivative a better inactivator of elastase than β-lactamase.[19]

Interestingly, 3-oxo-β-sultam **4** appears to inactivate elastase and possibly class C β-lactamase by acylation rather than sulfonylation (Scheme 7), despite hydrolysis of these compounds occurring with S−N fission. There is no elimination reaction of the inactivated enzymes to give a dehydroalanine residue from the active site serine. With elastase, but not β-lactamase, there is recovery of enzyme activity within a few hours, indicating acylation and formation of a labile ester rather than sulfonylation.

## Structure−Activity Relationships with β-Lactamase

β-Sultams are the sulfonyl analogues of β-lactams, and it is of interest to compare enzyme activity of their N-acyl derivatives with similarly substituted N-acyl β-lactams, **10**.

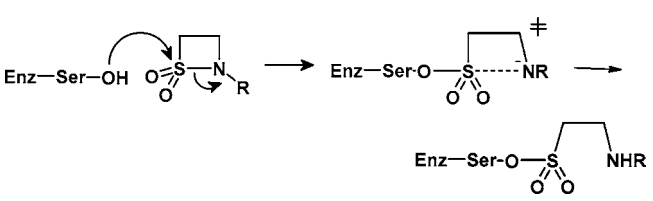


(10)

Class C β-lactamase P99 catalyzes the hydrolysis of monocyclic β-lactams **10** to give the ring-opened β-amidocarboxylic acid. The second-order rate constants, $k_{cat}/K_m$, for a series of substituted derivatives increase with electron-withdrawing substituents in the N-acyl residue and give a Bronsted $\beta_{lg}$ of −0.24. By contrast, the effect of changing the basicity of the leaving group in N-aroyl β-sultams on the rate of inactivation of β-lactamase gives a Bronsted $\beta_{lg}$ of −1.72. For comparison, the rate constants for the alkaline hydrolysis of the same series of N-aroyl β-sultams and N-aroyl β-lactams both generate similar $\beta_{lg}$ values of −0.7.

These differing Bronsted $\beta_{lg}$ values are indicative of different transition state structures for the two enzyme-catalyzed reactions. Taking the alkaline hydrolysis reactions of β-lactams and β-sultams as a reference indicates that there is a similar change in charge development on the leaving group nitrogen for both processes, probably suggesting a late transition state for formation of the respective intermediates with little or no C−N or S−N fission, respectively. By contrast, the Bronsted $\beta_{lg}$ of −1.72 for inactivation of β-lactamase by β-sultams indicates a large development of negative charge on the nitrogen leaving group compared with the effective positive charge of at least 0.7+ on nitrogen in the reactant.[35] It suggests that the leaving group is expelled as the amide anion and that S−N bond fission is complete or almost complete in the transition state (Scheme 8). The Bronsted $\beta_{lg}$ of −0.24 for the β-lactamase-catalyzed hydrolysis of β-lactams is much smaller than that seen with β-sultams and is indicative of little change in charge on the nitrogen leaving group on going from reactant to transition state. It suggests that C−N bond fission is not occurring in the transition state and that the rate-limiting step involves

**Scheme 8**



attack of the serine on the β-lactam carbonyl to form the tetrahedral intermediate.

Compared with the hydrolysis of N-acyl β-lactams by hydroxide ion, β-lactamase appears to cause a move to a transition state earlier along the reaction coordinate. This may be due to the stabilization brought about by the active site such as that of the oxyanion hole and compensation for the entropy loss in the bimolecular reaction. Conversely, compared with the hydrolysis of β-sultams by hydroxide ion, β-lactamase appears to cause a move to a transition state much later along the reaction coordinate. The enzyme appears to be using some of its catalytic machinery to facilitate the sulfonylation reaction but with nonideal geometry for the displacement step. The host of favorable noncovalent interactions, evolved by the enzyme to stabilize the transition state for the "natural" substrate, are not fully available to lower the activation energy for the β-sultams by the maximum amount. In terms of the Hammond postulate, this would be expected to lead to a later transition state as observed, whereas the hydrolysis of the β-lactams, which are more structurally related to the "natural" substrate, shows the opposite effect and exhibits an earlier transition state.

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